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The Predictive Link between Matrix and Metastasis

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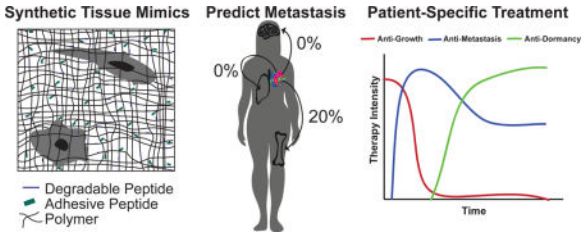
Abstract

Cancer spread (metastasis) is responsible for 90% of cancer-related fatalities. Informing patient treatment to prevent metastasis, or kill all cancer cells in a patient's body before it becomes metastatic is extremely powerful. However, aggressive treatment for all non-metastatic patients is detrimental, both for quality of life concerns, and the risk of kidney or liver-related toxicity. Knowing when and where a patient has metastatic risk could revolutionize patient treatment and care. In this review, we attempt to summarize the key work of engineers and quantitative biologists in developing strategies and model systems to predict metastasis, with a particular focus on cell interactions with the extracellular matrix (ECM), as a tool to predict metastatic risk and tropism.

Graphical abstract



Introduction

Metastasis, the spread of cancer cells from an initial tumor site to other areas of the tissue, or to other tissues entirely, is the cause of 90% of cancer-related deaths. Cancer does not metastasize randomly, rather each type of cancer exhibits a tissue-specific pattern of spread (called tropism) [3]. Some types, like colon [6] and ovarian cancer [7], are dictated by circulation patterns and anatomical proximity. But other types of cancer metastasize to distant organs independently of circulation. Prostate cancer metastasizes nearly exclusively

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to the bone [8], pancreatic cancer to the lung and liver, and other types, like breast and non-small cell lung cancer, spread to many tissues ([3], Figure 1).

By and far, evidence for what might control metastasis to certain tissues, but not others, has focused on the genetic determinants of the seed (the cancer cell) that dictate tissue selectivity (tropism) [9–13]. Here, we summarize engineered model systems to study the role of the soil (the extracellular matrix, ECM) in mediating metastasis. We discuss cell-ECM interactions and physical forces in guiding metastasis, with a focus on breast cancer. We discuss the community's ability to predict when and where cancer cells will metastasize, and speculate what these predictions mean for patient prognosis, surveillance, and drug treatment.

Predicting Invasion

Preceding eventual metastatic colonization at a secondary tissue, primary tumor cells must first invade through the basement membrane into the surrounding interstitial tissue. Biopsies are the gold standard for determining the local invasiveness of a patient's tumor upon diagnosis. There are many biomarkers, such as proteins, miRNAs, and copy number alterations that can identify a patient's tumor as invasive [14–17]. However, biopsies only provide one snapshot of a tumor, with limited ability to predict any stochastic changes in phenotype or mutations that may occur, thus limiting the future efficacy of a patient's drug treatment regimen. Recently, biologists and engineers have sought to understand how the ECM impacts the invasiveness of cancer, and if the ECM itself could be used as a predictive biomarker.

Matrix and/or cell stiffness is one such potential predictive marker. Recently a study found that mice with more mechanically compliant primary tumors had more aggressive relapsed tumors at the time of euthanasia [18]. Shear stress due to fluid flow also induces an epithelial to mesenchymal transition (EMT) in ovarian cancer, suggesting that more porous tumors are more metastatic [19]. On *in vitro* 2D surfaces, Leight *et al.* found that TGF-β1 induced invasion occurs only on rigid surfaces, thereby linking chemical and physical factors on the initiation of EMT [20]. A large study spearheaded by the National Cancer Institute (NCI) recently showed that physical differences (such as cell compliance, traction forces) are enough to distinguish between a malignant (MDA-MB-231) and normal (MCF-10A) cell line [21]. This study required massive efforts across many laboratories, so more sophisticated tools, perhaps those that rely on electrical impedance [22] instead of individual cell analysis, are required for high-throughput application.

In addition to stiffness, many ECM proteins have documented roles in augmenting or abrogating metastasis (for review, see [23]). In fact, Weinberg's Hallmarks of Cancer was recently updated to include the role of the ECM in each step of metastasis [24]. The potential for metastasis and the particular distant tissue site may be associated with an identifying "ECM signature" [25]. For example, using ECM-binding as a predictive marker showed that cancer cell binding to combinations of ECM proteins was sufficient to separate metastatic from non-metastatic cell lines [26]. While these are seminal studies, a consistent predictive biomarker of disease prognosis, such as the structure of the ECM, or perhaps an

individual or set of proteins within the ECM, has yet to be developed. This is imperative to translate understanding of the physical relationship between a cell and its ECM to clinical treatment.

Where will cancer metastasize?

Given what we know about the heterogeneous nature of tropism, how do we predict where a cell will metastasize? Ideally clinicians would be able to predict metastatic location with biomarkers in a tumor biopsy or in a blood draw, or with quick *in vitro* assays. An ideal *in vitro* platform would have controlled, decoupled properties, be highly reproducible, and be easily transferrable to different laboratories and clinics. In *in vitro* models, high invasion, motility, and proliferation are generally correlated with metastatic potential. One example recently shown by Sheetz and colleagues used simple control of mechanics to report that MDA-MB-231 variant cells proliferate *in vitro* on materials mechanically similar to the tissue to which they metastasize to *in vivo*, (Figure 2, [2]). This work was reproduced in a comparative study between ovarian and breast cancer cells (Figure 2, [4]). These examples suggest that physical features may filter for populations of breast cancer cells that exhibit enhanced proliferative ability at specific secondary tissue sites, and that biomaterial platforms with controlled mechanics could be used to identify this proliferative capacity *in vitro*.

Because the tissues recipient of breast cancer metastasis have striking differences in microenvironment composition, our lab and others have hypothesized that cell-matrix interactions may mediate tropism. We recently showed that integrin-mediated adhesion and motility phenotypes of breast cancer cells, compiled into a *phenotypic fingerprint* using a systems biology-like approach, can predict bone, brain, or lung metastasis in breast cancer (Figure 2, [1]). In contrast to tools that rely on genetics or fixed tumor tissue (Oncotype DX, MammaPrint, MetaSite Breast, and Prosigna), these engineering approaches use live cell interactions with the microenvironment and may reveal prognostic results missed by traditional approaches.

Tissue-Specific Drivers of Metastasis

Within breast cancer, the pathological subtype of the disease is correlated with different rates of metastasis to different tissues (Figure 1c, [5]). Bone metastases are by far the most common, occurring in 75% of all metastatic breast cancer patients. Brain metastases, on the other hand, are rare, occurring in roughly 15% of all patients, with ER^- negative and $HER2^+$ subtypes most commonly presenting. Metastasis to the lungs occurs in approximately 25% of metastatic patients, with the highest presentations in $HER2^+$, ER^-/PR^- breast cancer.

Pioneering genetic research has identified a subset of genetic markers associated with tissue-specific metastasis. These include COX2, ST6GALNAC5, EGFR, HBEGF [12] L1CAM and SERPINS [27], and GABA for brain metastasis [28]. Bone tropic populations overexpress genes that facilitate metastasis: CXCR4 promotes homing and extravasation, MMP1 facilitates invasion, CTGF and FGF5 aid angiogenesis, and IL11 and OPN are involved with osteolysis and remodeling the bone matrix [10]. Similar studies using lung-tropic human cells have identified up to 54 potential genes in mice, most prominently

IL13Rα2, SPARC, ID1, and VCAM1, to be involved in breast-to-lung metastasis [13]. These genetic markers have thus far provided the only possible predictive biomarkers in the primary tumor, and could serve as future therapeutic targets in the clinic.

To augment these genetic efforts, engineers are creating model systems, from simple-to-complex, to represent these tissue sites. These representations typically focus on healthy cells commonly found in these tissues, ECM protein composition, ECM stiffness, and tissue dimensionality. Hydrogels are popular tissue mimics because they recapitulate 3D tissue structure and physiological water content (Figure 3b) [29]. Natural hydrogels, such as collagen-based systems, elicit biochemical cues well, but over-sequester media proteins and cannot simultaneously recapitulate tissue stiffness and biochemical properties. While synthetic polymers can be independently tuned mechanically and chemically, they can be over-simplified, and more fundamental research on tissue properties is needed to make these models more tissue-specific. To combine the advantages of both, Beck et al. combined tunable, synthetic PEG gels with the common cancer model system Matrigel to investigate cell invasion in response to ECM stiffness [30]. Interestingly, they found very similar responses by both cancerous and normal cells, as both had reduced proliferation/morphogenesis in stiff environments. An analogous study used interpenetrating networks of Matrigel and alginate (both natural materials with very different properties), and somewhat in contradiction to the previous study, could force a malignant transformation in healthy breast cells via ECM stiffening [31]. Here, we briefly review approaches used to make these platforms more tissue-specific, and research that can be used to improve future tissue design parameters, with the hopes of uncovering a consensus of how these matrix features drive metastasis.

The most comprehensive study on tissue proteomics is available through the Proteinatlas (proteinatlas.org), which has annotated ~17,000 protein-coding genes across 32 tissues using both antibodies and RNAseq [32]. The brain ECM consists mainly of hyaluronan (HA) bound by lecticans and tenascin-R with some laminin, fibronectin, collagen IV, and heparin sulfate proteoglycans [33, 34]. Cancer cell binding to the brain ECM through integrins $\alpha_v\beta_3$ and $\alpha_v\beta_6$ [35] is also a possible biomarker of brain metastasis risk. The composition of the lung ECM is primarily elastin, collagen I, laminins, and glycosaminoglycans [36]. Upregulation and production of tenascin C, a transient glycoprotein, has been found along with lung metastases and is a sign of poor prognosis [13, 37]. Bone is a composite tissue, dominated by collagen I on the hard trabecular/cortical bone, and fibrillar collagen I, fibronectin, fibrinogen, and proteoglycans throughout the marrow [32].

Researchers are using a variety of techniques to quantify the rigidity of these tissue sites (Figure 3). Reports on the mechanical properties (stiffness) of brain tissue are primarily limited to elastography [38, 39], a technique not commonly performed on *in vitro* model systems. Obtaining human brain tissue for *ex vivo* mechanical testing is challenging, therefore indentation studies are limited to animal models, and report Young's moduli ranging from 1–2 kPa in Young's modulus [40]. The stiffness of the lung parenchyma has been measured using a variety of techniques in multiple animal models with a Young's modulus ranging from 2 to 8 kPa [41–43]. Trabecular and cortical bone is markedly stiff (~10GPa). Our lab recently reported the first physiological values for intact bone marrow

tissue stiffness (0.3–24.7 kPa), and we anticipate that improving the mechanical relevance of *in vitro* systems will elucidate mechanisms involved in breast-to-bone cancer progression [44].

A key defining characteristic of the skeleton in addition to its high stiffness is its *cyclic* mechanical nature - the primary regulator of bone cell function and remodeling [45], alongside increased pressure and fluid shear forces on cells within. When cancer cells arrive in the skeleton, they too are exposed to these cyclic signals, such as compression, hydrostatic pressure, and fluid shear stress. Tumor formation in the bone is inhibited in both breast and ovarian cancer during mechanical load [46, 47]. However, the magnitude of shear stresses and strains in the bone ECM are sufficient for driving malignancy in primary cancer cells *in vitro* [48], so understanding these relationships may help predict breast-to-bone metastasis.

Engineers have used these analyses as inspiration to create *in vitro* model systems of brain, bone, and lung tissue, to quantify interactions cancer cells with tissue-specific ECMs. *In vitro* models of brain include those based on HA [49], HA crosslinked with PEG [50], silk [51, 52], brain tissue slices, microfluidic devices, and some have begun using 3-D bioprinting [53, 54]. *In vitro*, cells that have been selected for lung metastasis have been found to grow optimally on intermediately stiff 3D hydrogel environments [2]. Decellularized tissues have been used to mimic bone ECM [55], as well as synthetic and silk-based scaffolds with either inverted colloidal geometry or electrospinning, functionalized with proteins found in bone [56, 57]. When implanted, they can capture circulating tumor cells [58].

Microfluidic systems can also capture features of cancer cell extravasation into bone-like matrices in the presence of local mesenchymal stem cells (MSCs) and endothelial cells [59]. One such lab developed a 3D metastasis model, called rMet, which is composed of ECM proteins characterizing of primary and secondary (bone) tissues sites of cancer metastasis [60]. Cancer cells that were highly invasive into their bone ECM were also highly metastatic to the bone in mice, demonstrating that an *in vitro* system can be predictive of *in vivo* behavior.

Combined with experimental results, predictive algorithms can be derived and incorporated into mechanics simulations. For example, Wang *et al.* incorporated osteocyte viability algorithms into their models simulating disuse and recovery to predict when bone tissue would undergo resorption or formation [61]. These types of systems could be used for metastasis studies, to predict how cancer cells will behave under a particular mechanical environment (e.g. will they be dormant, will they migrate elsewhere, fracture risk after a potential drug, etc).

The next step for model systems is to incorporate these matrices with vasculature [62, 63] for a more complete assessment of the metastatic cascade, and to translate these phenomenological behaviors into biomarkers useful for clinicians and patients at risk of metastasis, such as those identified *in vivo* [64].

Dormancy

Breakthrough research has revealed the existence of dormant tumor cells: metastasized cells quiescent at the metastatic site that may not proliferate, or “awaken” for several years, even decades, and are extremely difficult to detect and treat. Local stromal cells in the metastatic niche likely play a dominant role in paracrine activation of dormant cells, such as glial cells in the brain [65], and hepatocytes and non-parenchymal cells in the liver [66]. Vasculature plays a dual role, in some cases maintaining quiescence [67], and releasing the factors critical for re-activation in others [68]. Both Ghajar [69] and Aguirre-Guiso *et al.* [70] have proposed a therapeutic regimen to systemically treat possible dormant cells both during and after adjuvant therapy.

In vitro, cell adhesion to specific stromal-derived matrices [71], or substrates of particular stiffnesses [72] can permit dormancy. Specific combinations of bone marrow or hepatic stromal cells can induce breast cancer cell dormancy via cytokine secretion [66, 73]. For example, the Griffith lab used an engineered liver tissue to study stromal cell and ECM re-activation of dormant breast cancer cells in the liver [66]. They found that roughly half of infiltrated breast cancer cells were dormant, and not surprisingly, the more aggressive MDA-MB-231 cells were more sensitive to re-activation by non-parenchymal cells than the luminal MCF7s.

In combination with these systems, traditional culture platforms allow for selection of quiescent subpopulations, via isolating dormant cells after therapeutic administration [74] or from biomaterial systems that induce dormancy. By coupling these subpopulations with engineered microenvironments, it will be possible to accurately determine how therapy may be directed to induce dormancy and eradicate quiescent cells, preventing metastatic outgrowth altogether [69].

Predicting drug response for metastatic patients

Ideally, once metastasis risk is assessed, developing patient-specific drug regimes will follow. Anti-metastasis drugs include those targeted at vascularization, growth in certain microenvironments, cell-ECM binding, receptor kinases, and cancer stem cells (Figure 2d–f) [75]. *In vitro* model systems are being developed to test for drug response in more physiologically relevant microenvironments that could be tailored to specific tissue sites. A recent example is a 4D lung model, in which circulating tumor cells were as resistant to cisplatin in the model as they were *in vivo*, results not captured on 2D surfaces [76]. Similarly, combining ovarian cancer cells with fibroblasts in a representative ECM system recapitulated *in vivo* drug response [77]. These types of organotypic representations of tissue for drug screening are likely to become increasingly popular with the advent of patient-derived cultures [78]. These systems can be used with a more reductionist approach as well, e.g., ECM stiffness has been reported to be a powerful mediator of drug response for paclitaxel [79], ibuprofen [80], and sorafenib [81]. Ideally, the findings demonstrated here can be related to quantifiable, predictive biomarkers, informing drug treatment and monitoring.

Conclusions

Our discussion here was limited to experimental model systems to understand, predict, and treat metastatic spread. There is a keen need for computational methods to better inform and guide these experiments, such as how cells can resist chemotherapy treatment on the basis of likely heterogeneous drug distribution [82]. To our knowledge, no *in silico* approaches have been employed at point of care to predict metastatic spread. In addition, we suggest a need for computational experts to apply population-level survival models alongside kinetics to create a global map of cell trafficking from the primary tumor to eventual metastasis and growth in a secondary tissue site. This could include a probabilistic model of mutations inducing invasion, trafficking and dispersion throughout the body via circulation, and eventual rates of dormancy or rapid growth at a distant site. Experimental model systems are growing, and an increasing number of engineers are applying their expertise to cancer metastasis. Model system improvements include increasing their throughput, including patient-derived cells, and translating their predictive findings into clinically useful biomarkers.

Acknowledgments

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Highlights

- *In vitro* systems are needed to predict if, when, and where a tumor will metastasize.
- The ECM is a major driver of metastasis to specific tissue destinations.
- Mechanical forces play a major role in driving invasion and metastatic outgrowth.
- Predictive tools need to be moved toward clinical use, e.g. biomarkers.

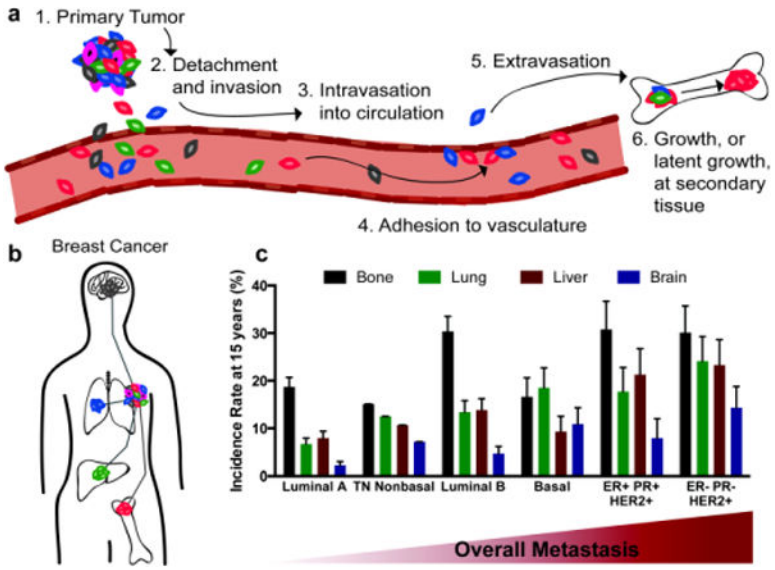

Figure 1.

Breast cancer metastatic cascade. a) Overview of metastatic process. We focus on steps 2 and 6 in this review. b) Common sites of breast cancer metastasis, adapted from Barney *et al* [1]. c) Through a large clinical study, Kennecke *et al.* found correlations with breast cancer subtype and frequency of metastases found at specific tissue sites, data reproduced from [5]. (ER=estrogen receptor, PR=progesterone receptor, and HER2=human epidermal growth factor receptor 2, and TN=triple negative)

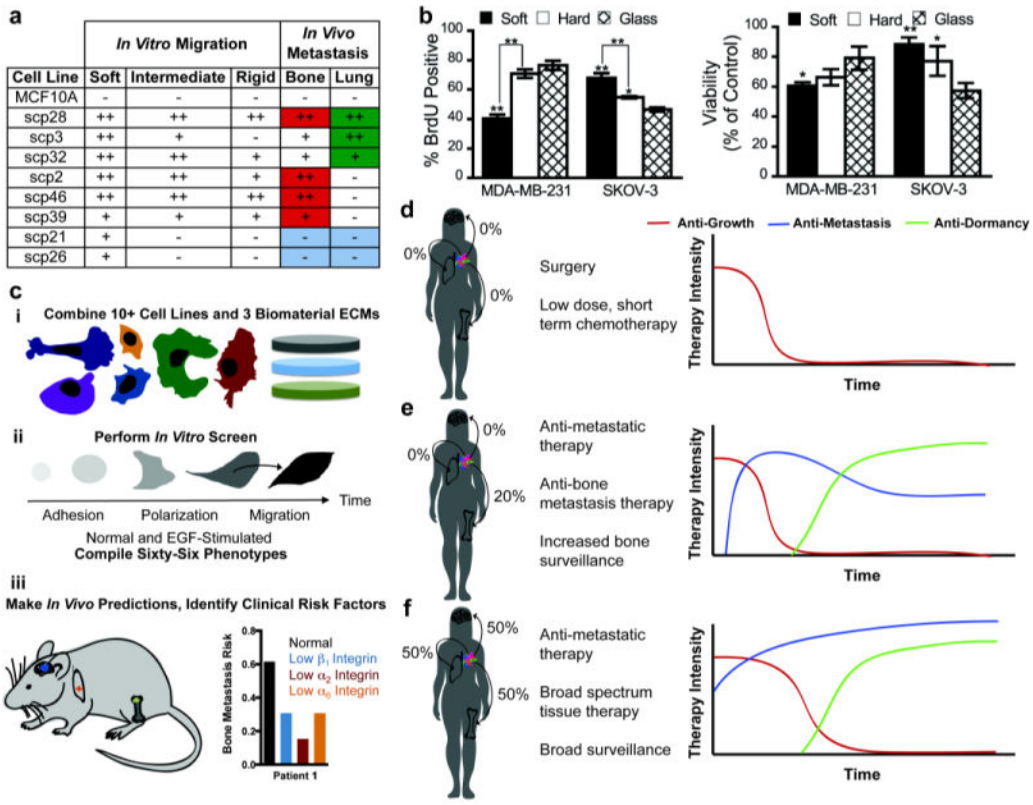

Figure 2.

Predicting metastasis and potential impact on therapy. a) Cells that metastasized to bone and lung *in vivo* shared similar *in vitro* motility characteristics, adapted from Kostic *et al.* [2]. b) Mechanosensitivity can be used to distinguish between breast cancer metastatic cells (MDA-MB-231) and ovarian cancer metastatic cells (SKOV-3), from McGrail *et al.* [4]. c) Barney et al. developed an *in vitro* screen that used integrin-binding to predict *in vivo* metastasis to the bone, brain, and lung, as well as identify integrin subunits as tissue-specific risk factors [1] ADD REFERENCE. d–f) One can envision that these types of predictive screens could impact therapy for patients with different risk factors for developing metastatic disease. Patients with low risk of metastasis could be shielded from harsh treatment modalities (d), whereas patients at risk for metastasis (e, f) could be treated aggressively to help prevent metastasis or relapse.

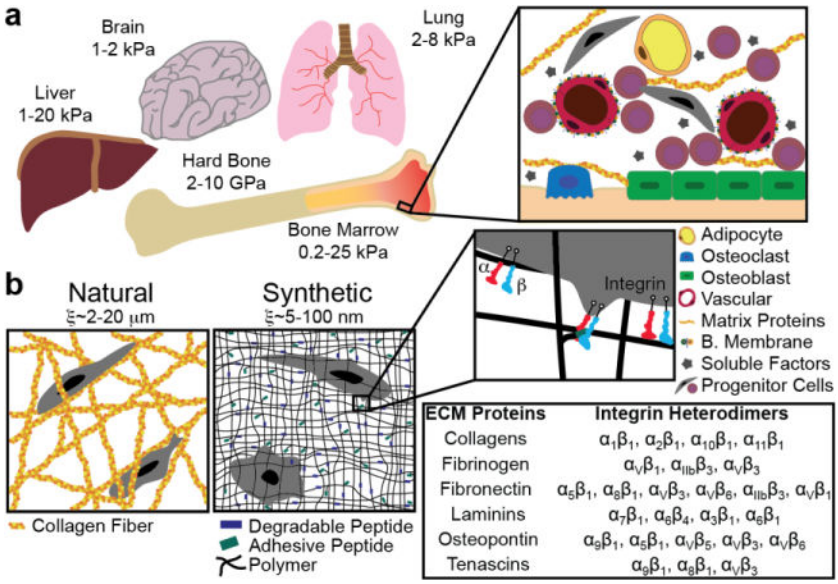

Figure 3.

In vitro models of the metastatic niche. a) Common sites of breast cancer metastasis and their published stiffnesses (Young's modulus). These sites consist of tissue-specific cell types that are part of the metastatic niche. b) These tissues can be represented by natural protein fiber gels or synthetic polymer materials. Polymer matrices can be modified to allow for tissue-specific cell-ECM interactions (via integrin binding) by including oligopeptides responsible for the integrin-binding domains of full-length ECM proteins typically found in these tissues.